

HTS – where next?

After approximately a decade of maturation and a three orders of magnitude increase in capabilities, High Throughput Screening (HTS) is now an established discipline within pharmaceutical discovery. But opposing stresses exist within the screening world – the resolution of which will significantly affect the path along which the HTS discipline will develop.

Those of us working in pharmaceutical discovery during the 1990s witnessed a massive increase in the industry's ability to screen large numbers of compounds against therapeutic disease area targets. The purpose of this exercise was the identification of appropriate target specific biological activity within the compound file. Once identified, such compounds formed the basis of medicinal chemistry-led lead optimisation programmes that would ultimately deliver compounds into clinical development.

Historically, *in vitro* biological screening was, probably correctly, regarded very much as a service function for the chemistry teams. Its practitioners were expected to quickly turn around requests for data from the project chemists in the drive to identify biological activity and further refine the team's knowledge of the interaction of a small number of chemical classes with the target of interest.

Biochemical screening, as opposed to *in vitro* pharmacology screening, was characterised as an entirely manual process in which one full-time equivalent was able, depending upon the screen protocol, to screen at most a few hundred file compounds per week. Test compounds arrived in the laboratory as solids in bottles, the contents of which needed to be dissolved in an appropriate

vehicle before being transferred to a reaction vessel, typically a 96-well microplate to which the appropriate biochemical reagents were added. The screen protocols usually required one or more separation steps, for example centrifugation, phase extraction, filtration or precipitation, which were not conducive to operation at high levels of intensity. At the conclusion of the screen, the effect of the file compound on the target would be measured. The instrumentation available frequently did not support the use of microplates; therefore requiring an additional vessel transfer operation. Significantly, the entire process was not integrated and all information concerning the test compound identity, concentration and lot number, as well as the results from the screen itself, had to be collated manually, frequently involving the manual entry of data. Typically during the lifetime of a typical project, its screen would test 5,000-20,000 compounds over several years. At its conclusion, it would have screened some 2.5% to 20% of the total available compound file.

With the benefit of hindsight, the scenario described above seems at best a quaint and vaguely amusing description of times past. However, it should be remembered that the screening groups were achieving their core purposes as a service to chemistry-led lead identification programmes.

By Dr Mark Beggs

High throughput screening

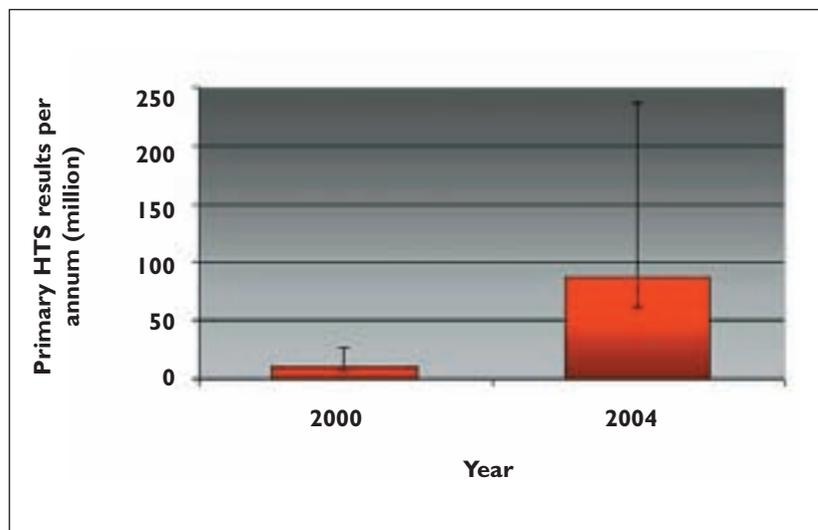


Figure 1
Present performance levels and future expectations for uHTS. Data shown represent the current annual results generated by primary HTS screening for a typical pharmaceutical company in 2000 and projected expectations in five years time. Error bars represent maxima and minima performance levels

During the last decade the position has changed radically and screening has variously acquired the epithet ‘high throughput’ (HTS) or ‘ultra high throughput’ (uHTS). Today’s leading edge uHTS practitioners are claiming peak throughput rates of 10,000-100,000 compounds within a 24-hour day – some 2-3 orders of magnitude above the capabilities of the early 1990s! A major pharma’s

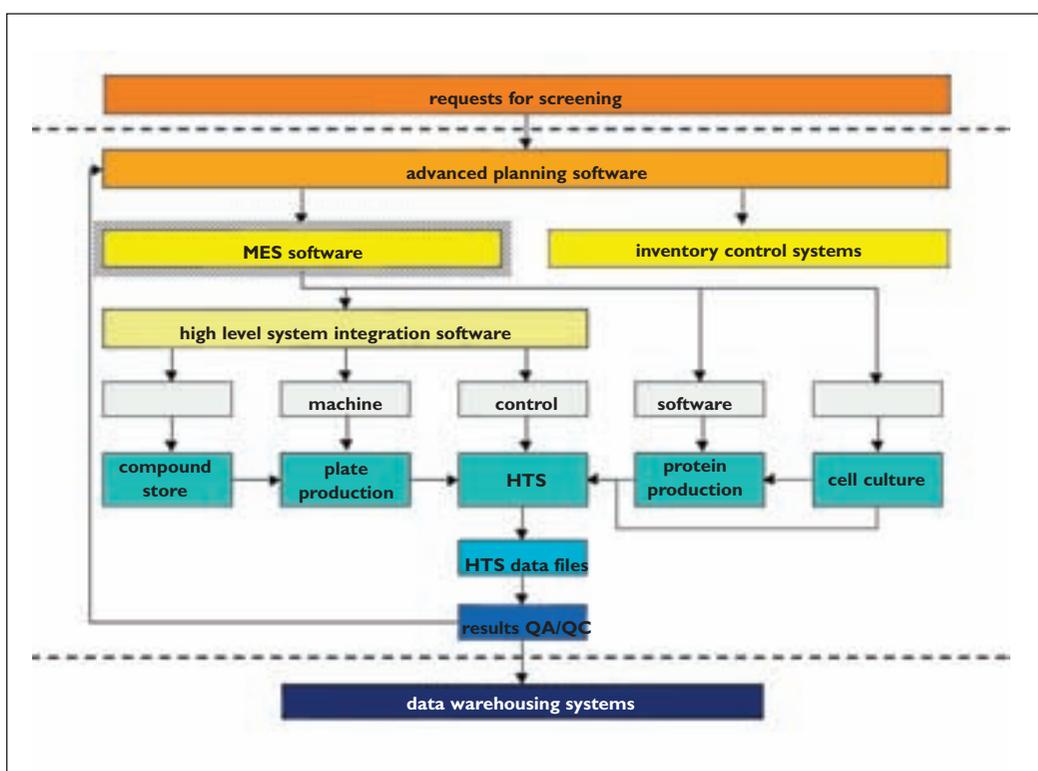
screening facility typically mounts 20-35 campaigns per annum against an entire file collection of 100,000 to 500,000 compounds; thereby generating some 2-18 million primary screening results per annum.

The drivers for such a major overhaul in the industry’s screening capabilities were several-fold. Firstly was the belief that it was desirable to screen the entire compound file rather than a subset whose selection was based on human intuition. Indeed, one of the basic tenets behind empirical screening is that it will lead to the identification of hitherto unknown structure-activity relationships.

Secondly, the development of ‘HTS friendly’ assay technologies including among others Scintillation Proximity Assay², HTRF³ and FCS⁴ have obviated the need for complex screen protocols and greatly improved both throughput rates and data quality.

Thirdly, microplate-based instrumentation and workstations were developed which could be linked together either using a robotic platform or by human operators. Finally, the widespread deployment of cheap computing power has led to the integration of the various HTS elements to the point that data capture, compound information and QA/QC processes can be handled electronically in a seamless manner.

Figure 2
System control architecture required for intensive uHTS facility operation. Multiple layers of controlling software will be needed to permit a high response mode of operation. Some interfaces have been omitted for simplification



Before the industry congratulates itself too much on a 'job well done' we need to examine the realities that lie behind today's uHTS operations. The core business purpose of a uHTS function could be expressed simply as "...to screen compounds in support of overall Discovery objectives..." While few would dispute this statement publicly, many of the activities that occur within screening groups are, at best, a diversion or, at worst, counter productive to the core objective. What are these activities, why do they occur and where should the focus lie?

Rather than review the available options in HTS assay technology or automation, which have been comprehensively described elsewhere¹, this article addresses the pressures for change confronting lead identification and the resulting organisational challenges facing the future management of HTS.

Has HTS automation delivered so far?

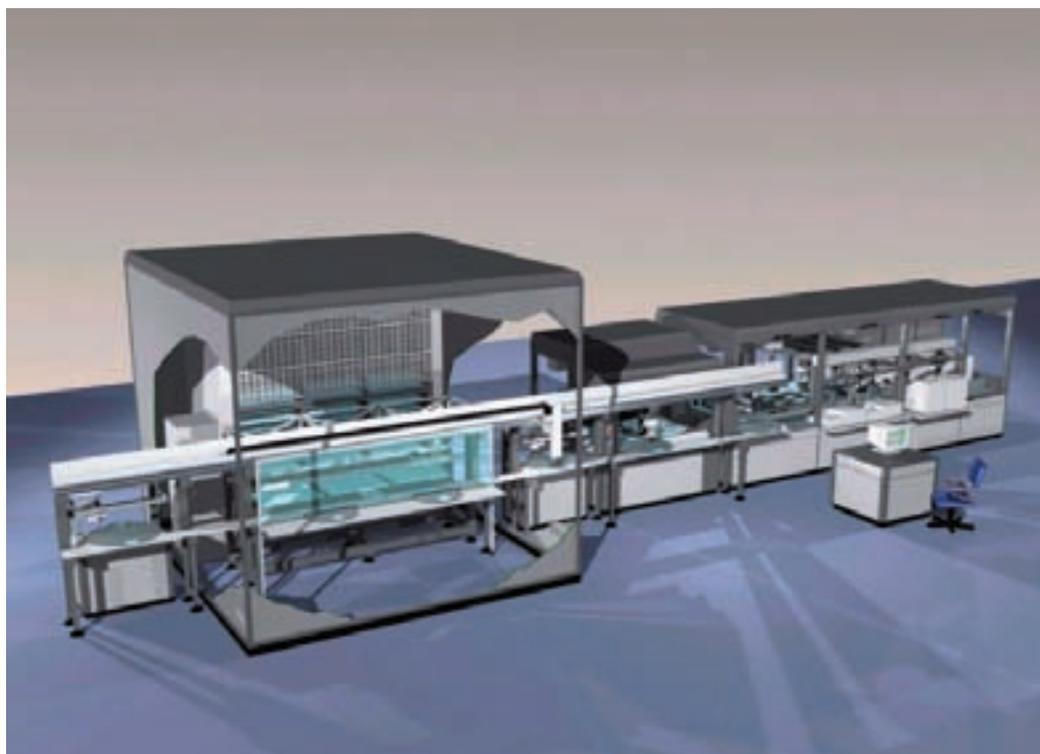
One of the benefits that accrued to the HTS practitioner during the 1990s expansion phase was that the profile of HTS was raised significantly. The large investments made in new assay technologies, compound management and automation placed the HTS teams under a veritable technology spotlight. Those with an aptitude for HTS automation found themselves included on corporate PR tours and invited to present talks at international symposia. Investments in new automation systems were made, in many cases, without consideration of the overall utility of the selected system in the context of the requirements of the screening group. This was especially true when the real output of the new automation platform became apparent. Such systems operate in conjunction with regular screening staff working a single eight-hour shift. Thus, the real output of such platforms is predicated upon not just by its steady state output but also by factors such as biochemical reagent stability.

The deployment of complex integrated systems required the acquisition of specialised support skill sets, the existence of whom diverted headcount away from the core screening team. Finally, experience has shown that the time taken to reconfigure automation platforms for the next screen often means that campaign start dates are pushed backwards. The reality to date is that in-house system integrations have often consumed more resource than the benefits they deliver owing to the complexity of the system itself and higher level integrations with other systems which may themselves be subject to frequent

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Figure 3

Higher levels of hardware integration will break down traditional organisational divisions and allow the rapid turnaround of primary screen hits to potency ranked lead series. The figure shows an automated liquid store integrated with plate preparation, cherry picking and uHTS modules



change. Is it now time to consider outsourcing major future automation and software initiatives to organisations who are measured on, and paid by, result milestones?

Why miniaturise?

The frustrations encountered with the productivity of automated screening systems working with 96-well microplates led to a logical drive towards higher density microplate formats. At the point of writing the 384-well microplate represents the most pragmatic balance between ease of use and benefit of throughput. Work continues in many groups to develop the 1536-well microplate as a practicable option for screening. Other groups are investigating ultra miniaturisation strategies that employ micro-fabrication techniques to assemble devices able to perform screens using sub-microlitre to nanolitre liquid handling and instrumentation techniques.

The other often-cited reason for investing in miniaturisation technologies is one of reagent cost. This argument usually resolves further into the problem of securing adequate amounts of 'in-house'-produced biological materials such as recombinant proteins, receptors or whole cells to support the screening of a large compound file. The provision of up to 1012 cells

expressing recombinant protein adequate for a 384-well microplate-based screening campaign is by no means an unrealistic expectation for a well-equipped cell culture facility able to support the provision of both adherent and non-adherent cells. Indeed, in cases of 'best practice uHTS', this level of productivity is already being realised.

It is the contention of this article that the management issues involved in securing adequate amounts of cell culture resource for screening support is a more tractable issue than the high financial, resource and risk strategy of acquisition of 'lab on a chip' assay technologies. The management challenge is therefore to establish service level agreements for the timely provision of small scale (validation) and subsequently large scale (screening) batches of cells for reagent production and to ensure that both the cell production and uHTS teams are aware of each other's lead times, priorities and requirements. This is especially true in cases of cell-based screens where the production and delivery of the cells needs to be co-ordinated with their use in the uHTS screen itself. In cases where this has been made to work, a key factor for success is bringing the two disciplines under a single set of management.

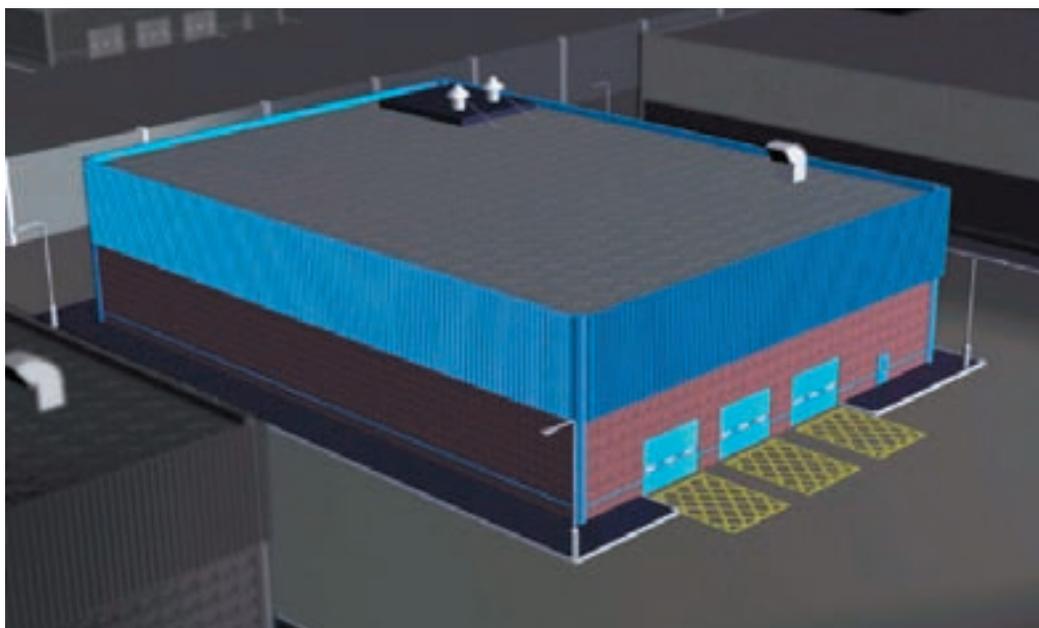
HTS – where next ?

The HTS benchmarks are moving again. Expectations of the major players are that they will be able to mount between 50 and 100 screening campaigns per annum against a compound file collection of 0.5 to 1.5 million compounds. This will result in the generation of between 25 to 150 million results per annum. Expressed another way, assuming that uHTS continues to work on a 40-hour single shift basis, the 150 million results per annum represents a mean rate of result generation of 1,200 datapoints per minute. Peak rates of production would presumably be rather higher! The above scenario reflects an expectation that productivity will increase between 9 and 13-fold above current levels (Figure 1). Some leading edge practitioners of HTS are already planning for this eventuality.

Clearly, should the above scenario become reality, the prosecution of screening is likely to become a rather different world from that today. A 9 to 13-fold increase in the intensity of operation will require significant organisational change. It will be necessary to assemble the related functions of assay development/adaptation cell culture, protein purification, compound management, HTS, automation and IT support functions into an integrated management structure whose focus is the delivery of quality results at an intensity of operation that matches the overall business objective⁴. Staffing and management skills and levels will have to be adapted to suit. It is likely that the facility will operate on a multiple shift basis. The introduction of new screening targets, projected at 1 to 2 new 'live' targets per week, will need to be tightly managed as the supply of materials and availability of hardware and key staff will need to be controlled. Resource management approaches will need to be adopted to account for 'real world' events such as hardware failures. Machine downtime will need to be factored into its overall specification and the output of automation systems comprehended not in terms of "the best it can ever do multiplied by 365 days" but instead in terms of response time to deliver a request for work. Advanced planning and optimisation systems, including demand management capabilities will need to be configured and deployed (Figure 2). Collaborative planning, both with internal and external suppliers, will become the norm as will the use of best practice procurement techniques. These are all techniques that have been deployed within manufacturing industry, including pharmaceuticals. However, the key to success

High throughput screening

Figure 4
A future drug discovery facility will utilise production style rather than campus style architecture



is to comprehend and articulate the science in terms of manufacturing process.

The cultural changes required to support these behavioural patterns should not be ignored. The production-focused facility needs to interface with the more fluid and unregulated world of discovery research yet, in its own operation needs to be more rigorous and quantifiable. A key area is the interface of assay development with assay adaptation. Here the screen protocol parameters need to be defined to the extent that key sensitivities are articulated and tolerances defined. Once these are understood, it can then be determined whether the screen protocol, in its present format, is suitable for operation on the facility's hardware.

Clearly a production-based screening facility will require a significant investment in hardware (Figure 3) to cope with the planned intensity of use. However, merely investing heavily in hardware, no matter how state-of-the-art, is unlikely to deliver the desired returns unless the organisational issues are addressed at the same time.

The future

The next few years will be critical for the future success of the HTS discipline. One of two avenues is open to the management of HTS: "do more the way we do it now" or restructure along the lines of a task-orientated production facility. If the industry carries on with today's behaviour-

al model where "interesting science and technology" diverts key resource away from the core purpose it is unlikely that the desired future levels of productivity can be sustained. An appropriately structured 'Drug Discovery Factory' facility⁵ would deliver the output required from an increasingly more demanding industry (Figure 4). Of course, such techniques have equal applicability in other 'screen intensive' areas of pharma including genotyping and toxicology functions. It will be interesting to watch in which discipline these techniques are first adopted. Either way, HTS is in for an interesting time over the next few years.

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Dr Mark Beggs leads TAP's Integrated Discovery initiative in which role he provides consultancy on complex screening systems design. Mark is a bio-chemist/enzymologist with 10 years' experience within Pharma. He was formally Director of HTS at Johnson & Johnson's Janssen Research Foundation, and prior to that was involved in HTS system operation and assay development at Zeneca. Previous to this, he completed his post-doctoral research in endocrinology and metabolic regulation at Glaxo's (GSK's) Research Triangle Park facility. Mark is a Council Member of the Society for Biomolecular Screening.